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(54) Title: A METHOD FOR IDENTIFICATION, ISOLATION AND PRODUCTION OF ANTIGENS TO A SPECIFIC PATHOGEN

(57) Abstract: Described is a method for identification, isolation and production of hyperimmune serum-reactive antigens from a specific pathogen, a tumor, an allergen or a tissue or host prone to autoimmunity, said antigens being suited for use in a vaccine for a given type of animal or for humans, which is characterized by the following steps: - providing an antibody preparation from a plasma pool of said given type of animal or from a human plasma pool or individual sera with antibodies against said specific pathogen, tumor, allergen or tissue or host prone to auto-immunity, - providing at least one expression library of said specific pathogen, tumor, allergen or tissue or host prone to auto-immunity, - screening said at least one expression library with said antibody preparation, - identifying antigens which bind in said screening to antibodies in said antibody preparation, - screening the identified antigens with individual antibody preparations from individual sera from individuals with antibodies against said specific pathogen, tumor, allergen or tissue or host prone to auto-immunity, - identifying the hyperimmune serum-reactive antigen portion of said identified antigens and which hyperimmune serum-reactive antigens bind to a relevant portion of said individual antibody preparations from said individual sera and - optionally isolating said hyperimmune serum-reactive antigens and producing said hyperimmune serum-reactive antigens by chemical or recombinant methods.

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A METHOD FOR IDENTIFICATION, ISOLATION AND PRODUCTION OF ANTIGENS TO A SPECIFIC PATHOGEN

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Abstract of **WO02059148**

Described is a method for identification, isolation and production of hyperimmune serum-reactive antigens from a specific pathogen, a tumor, an allergen or a tissue or host prone to autoimmunity, said antigens being suited for use in a vaccine for a given type of animal or for humans, which is characterized by the following steps: - providing an antibody preparation from a plasma pool of said given type of animal or from a human plasma pool or individual sera with antibodies against said specific pathogen, tumor, allergen or tissue or host prone to auto-immunity, - providing at least one expression library of said specific pathogen, tumor, allergen or tissue or host prone to auto-immunity, - screening said at least one expression library with said antibody preparation, - identifying antigens which bind in said screening to antibodies in said antibody preparation, - screening the identified antigens with individual antibody preparations from individual sera from individuals with antibodies against said specific pathogen, tumor, allergen or tissue or host prone to auto-immunity, - identifying the hyperimmune serum-reactive antigen portion of said identified antigens and which hyperimmune serum-reactive antigens bind to a relevant portion of said individual antibody preparations from said individual sera and - optionally isolating said hyperimmune serum-reactive antigens and producing said hyperimmune serum-reactive antigens by chemical or recombinant methods.

Description of **WO02059148**

A method for identification, isolation and production of antigens to a specific pathogen

The invention relates to a method for identification, isolation and production of antigens to a specific pathogen as well as new antigens suitable for use in a vaccine for a given type of anir

Vaccines can save more lives (and resources) than any other medical intervention. Owing to world-wide vaccination programmes the incidence of many fatal diseases has been decreased

Although this notion is valid for a whole panel of diseases, e. g. diphtheria, pertussis, measles and tetanus, there are no effective vaccines for numerous infectious disease including most v the fact that infectious diseases, rather than cardiovascular disorders or cancer or injuries remain the largest cause of death and disability in the world.

Several established vaccines consist of live attenuated organisms where the risk of reversion to the virulent wild-type strain exists. In particular in immunocompromised hosts this can be ε

Whilst there is no doubt that the above vaccines are valuable medical treatments, there is the disadvantage that, due to their complexity, severe side effects can be evoked, e. g. to antigens

Some widely used vaccines are whole cell-vaccines (attenuated bacteria or viruses (e. g. Bacille Calmette-Guerin (BCG) (tuberculosis), Measles, Mumps, Rubella, Oral Polio Vaccine (Sa

A vaccine can contain a whole variety of different antigens. Examples of antigens are whole-killed organisms such as inactivated viruses or bacteria, fungi, protozoa or even cancer cells. conjunction with majorhistocompatibility complex(MHC). B-cells can recognize linear epitopes as short as 4-5 amino acids, as well as three dimensional structures (conformational epitop Intermediate cell types may also be involved. Only effector cells with the appropriate specificity are activated in a productive immune response. The adjuvant may also locally retain antig

Antigen presenting cells belong to the innate immune system, which has evolved as a first line host defence that limits infection early after exposure to microorganisms. Cells of the innate antigenic structures, including peptides, in the case of T-cells and peptides as well as three-dimensional structures in the case of Bcells. The adaptive immune system is much more specifi the adaptive immune system and thus trigger specific immune responses leading to clearance of the intruders. In sum, cells of the innate immune system and in particular APCs play a criti

The antigens used for such vaccines have often been selected by chance or by easiness of availability. There is a demand to identify efficient antigens for a given pathogen or-preferably-a

It is therefore an object of the present invention to comply with these demands and to provide a method with which such antigens may be provided and with which a practically complete s

Therefore, the present invention provides a method for identification, isolation and production of hyperimmune serum-reactive antigens from a specific pathogen, a tumor, an allergen or a or host prone to auto-immunity, providing at least one expression library of said specific pathogen, a tumor, an allergen or a tissue or host prone to auto-immunity, screening said at least c identified antigens which hyperimmune serum-reactive anti gens bind to a relevant portion of said individual antibody preparations from said individual sera and optionally isolating said I

This method is also suitable in general for identifying a practically complete set of hyperimmune serum-reactive antigens of a specific pathogen with given sera as antibody sources, if at l humans, which is characterized by the following steps: providing an antibody preparation from a plasma pool of said given type of animal or from a human plasma pool or individual sera from individuals with anti bodies against said specific pathogen, identifying the hyperimmune serum-reactive antigen portion of said identified antigens which hyperimmune serum-reacti screening and identification steps, if at least5% of the hyperimmune serum-reactive antigens have been identified in the repeated screening and identification steps only, until less than 5 %

The method according to the present invention mainly consists of three essential parts, namely1. identifying hyperimmune serum sources containing specific antibodies against a given pat and not only hyperimmune serum-reactive, but also widely immunogenic (i. e. that a lot of individual sera react with a given antigen). With the present method it is possible to provide a s

Completeness of the antigen set of a given pathogen within the meaning of the present invention is, of course, dependent on the completeness of the expression libraries used in the presen

A serum collection used in the present invention should be tested against a panel of known antigenic compounds of a given pathogen, such as polysaccharide, lipid and proteinaceous com with acute disease with different manifestations (e. g. S. aureus sepsis or wound infection, etc.), 3. With no specific antibodies at all (as negative controls): 5-8 months old babies who lost

In the antigen identification programme for identifying a complete set of antigens according to the present invention, it is preferred that said at least three different expression libraries are serumreactive antigens by using only one or two different expression libraries, this might in many cases not finally result in the identification of a complete set of hyperimmune serum-rea

According to the present invention also serum pools or plasma fractions or other pooled antibody containing body fluids are "plasma pools".

An expression library as used in the present invention should at least allow expression of all potential antigens, e. g. all surface proteins of a given pathogen. With the expression libraries case of extracellular pathogens preferably a protein preparation containing surface proteins of said pathogen obtained from said pathogen grown under defined physiological conditions (s

While the genomic approach has the potential to contain the complete set of antigens, the latter one has the advantage to contain the proteins in their naturally state i. e. including for insta al., 1994. Especially preferred are expression libraries representing a display of the genetic set of a pathogen in recombinant form such as in vitro translation techniques, e. g. ribosomal di

Ribosome display is an established method in recombinant DNA technology, which is applicable for each specific pathogen for the sake of the present invention(Schaffitzel et al, 1999). B and expression via exported proteins are also preferred as bacterial surface expression library (Forrer et al., 1999; Rodi andMakowski, 1993; Georgiou et al., 1997).

The antigen preparation for the first round of screening in the method according to the present invention may be derived from any source containing antibodies to a given pathogen. Prefer especially shown for the preferred embodiments of the present invention.

Preferably the expression libraries are genomic expressionli- braries of a given pathogen, or alternativelym-RNA, libraries.

It is preferred that these genomic or m-RNA libraries are complete genomic or m-RNA expression libraries which means that they contain at least once all possible proteins, peptides or p

Preferably, the method according to the present invention comprises screening at least a ribosomal display library, abacte- rial surface display library and a proteome with the antibody pre including post-translational modifications, processing, etc. which are not obvious from the DNA sequence. I
The method according to the present invention may be applied to any given pathogen. Therefore, preferred pathogens are selected from the group of bacterial, viral, fungal and protozoan more complicated for complex (eukaryotic) organisms having large genomes.

However, also such large genomic libraries of higher organism pathogens may well be analyzed with the method according to the present invention, at least in a faster and more reliable w

Preferred pathogens to be analyzed or which antigens are to be extracted, respectively, include human immunedeficiency virus (HIV), hepatitis A virus (HAV), hepatitis B virus (HBV), h Chlamydia pneumoniae (C. pneumoniae), Chlamydia trachomatis (C. trachomatis), Mycobacterium tuberculosis(M. tuberculosis), Mycobacterium leprae (M.leprae), Streptococcus pneum Bacillus anthracis (B. anthracis), Vibrio cholerae (V. cholerae),
Borrelia burgdorferi (B. burgdorferi), Plasmodium sp., fungal diseases such as Pneumocystis carinii, Aspergillussp., Cryptococcussp., Candida albicans or parasitic infections such as asca

The method according to the present invention is most applicable for bacteria, worms or Candida.

As a model organism for the present application Staphylococcus aureus has been chosen to demonstrate the applicability and efficacy of the method according to the present invention. Es

It was surprising that the method according to the present invention allows an efficient and fast biological screening of a given pathogen, especially in view of the fact that only a small fra portion is directed against non-protein antigens, such as teichoic acid, so that only a total of 0.1% or less of the antibodies are directed to proteinaceous antigens.

One of the advantages of using recombinant expression libraries, especially ribsome display libraries and bacterial surface display libraries, is that the identified hyperimmune serum-react DNA technology or cloning steps necessary.

The hyperimmune serum-reactive antigens obtainable by the method according to the present invention may therefore be immediately finished to a pharmaceutical preparation, preferably

Preferably, the pharmaceutical preparation containing the hyperimmune serum-reactive antigen is a vaccine for preventing or treating an infection with the specific pathogen for which the

The pharmaceutical preparation may contain any suitable auxiliary substances, such as buffer substances, stabilisers or further active ingredients, especially ingredients known in connecti

A preferable carrier/or excipient for the hyperimmune serum-reactive antigens according to the present invention is a immunostimulatory compound for further stimulating the immune re combinations thereof.

The polycationic compound (s) to be used according to the present invention may be any polycationic compound which shows the characteristic effects according to the WO 97/30721. Pr

(1983)). Especially preferred are substances like polylysine, polyargininc and polypeptides containing more than 20%, especially more than50% of basic amino acids in a range of more th

These polycationic compounds may be produced chemically or recombinantly or may be derived from natural sources.

Cationic (poly) peptides may also be anti-microbial with properties as reviewed in Ganz et al, 1999; Hancock, 1999. These (poly) peptides may be of prokaryotic or animal or plant origin

Ganz et al., 1999; Simmaco et al., 1998). Peptides may also belong to the class of defensins (Ganz, 1999; Ganz et al., 1999).

Sequences of such peptides can be, for example, be found in the
Antimicrobial Sequences Database under the following internet address: http ://www. bbcm. univ. trieste.it/-tossi/paa2. html
Such host defence peptides or defensives are also a preferred form of the polycationic polymer according to the present inven tion. Generally, a compound allowing as an end product acti

Especially preferred for use as polycationic substance in the present invention are cathelicidin derived antimicrobial peptides or derivatives thereof (International patent applicationPCT/EI

Polycationic compounds derived from natural sources include HIV
REV or HIV-TAT (derivedcationic peptides, antennapedia peptides, chitosan or other derivatives of chitin) or other peptides derived from these peptides or proteins by biochemical orrec
the substitution or modification of the natural amino acids by amino acids which are not among the 20 standard amino acids. Moreover, further cationic residues may be introduced into su

It is therefore possible to use such cathelin molecules as efficient adjuvants in vaccine formulations with or without further immunactivating substances.

Another preferred polycationic substance to be used according to the present invention is a synthetic peptide containing at least 2 KLK-motifs separated by a linker of 3 to 7 hydrophobic a

Immunostimulatory deoxynucleotides are e. g. neutral or artificial
CpG containing DNA, short stretches of DNA derived from non-vertebrates or in form of short oligonucleotides (ODNs) containing non-methylated cytosine-guanine di-nucleotides (CpG

Neuroactive compounds, e. g. combined with polycationic substances are described in WO 01/24822.

According to a preferred embodiment the individual antibody preparation for the second round of screening are derived from patients with have suffered from an acute infection with the g
hyperimmune serum-reactive antigens to the given pathogen.

It is important that the second screening with the individual antibody preparations (which may also be the selected serum) allows a selective identification of the hyperimmune serum-reac

Therefore, preferably at least 10 individual antibody preparations (i. e. antibody preparations (e. g. sera) from at least 10 different individuals having suffered from an infection to the chos
preferably at least 30, especially at least 50 individual antibody preparations, identification of hyperimmune serum-reactive antigen is also selective enough for a proper identification.Hyp

Therefore, the relevant portion of the hyperimmune serum-reactive antibody preparation according to the method of the present invention should preferably be at least 10, more preferred a

According to a preferred embodiment of the present invention, the sera from which the individual antibody preparations for the second round of screening are prepared (or which are used

Preferably, some are selected with a total IgA titer above 4000
U, especially above 6000 U, and/or an IgG titer above 10 000 U, especially above 12 000 U (U = units, calculated from the OD reading at a given dilution) when whole organism (total lys

According to the demonstration example which is also a preferred embodiment of the present invention the given pathogen is a
Staphylococcus pathogen, especially Staphylococcus aureus and
Staphylococcus epidermidis. Staphylococci are opportunistic pathogens which can cause illnesses which range from minor infections to life threatening diseases. Of the large number of
Staphylococci at least 3 are commonly associated with human disease: S. aureus, S. epidermidis and rarely S. saprophyticus (Crossley and Archer, 1997). S. aureus has been used within th
to induce multi-drug resistance. For that reason medical treatment against Staphylococcal infections cannot rely only on antibiotics anymore.

Therefore, a tactic change in the treatment of these diseases is desperately needed which aims to prevent infections. Inducing high affinity antibodies of the opsonic and neutralizing type b

Every human being is colonized with S. epidermidis. The normal habitats of S. epidermidis are the skin and the mucous membrane.

The major habitats of the most pathogenic species, S. aureus, are the anterior nares and perineum. Some individuals become permanent S. aureus carriers, often with the same strain. The c
nosocomial Staphylococci. These bacteria have an innate adaptability which is complemented by the widespread and sometimes inappropriate use of antimicrobial agents. Therefore hospi
staphylococci may be untreatable by antibiotics. In addition to its adverse effect on public health, antimicrobial resistance contributes to higher health care costs, since treating resistant inf

Moreover, even with the help of effective antibiotics, the most serious staphylococcal infections have 30-50% mortality.

Staphylococci become potentially pathogenic as soon as the natural balance between microorganisms and the immune system gets disturbed, when natural barriers (skin, mucous membrai
related to medical devices, such as intravascular and percutan catheters (endocarditis, sepsis, peritonitis), prosthetic devices (septic arthritis, osteomyelitis). S. epidermidis causes diseases
association with the use of intravascular device. The increase in incidence is related to the increased used of these devices and increasing number of immunocompromised patients.

Much less is known about S. saprophyticus, another coagulasenegative staphylococci, which causes acute urinary tract infection in previously healthy people. With a few exceptions these

The pathogenesis of staphylococci is multifactorial. In order to initiate infection the pathogen has to gain access to the cells and tissues of the host, that is adhere. S. aureus expresses-surfa
staphylococci use are the secreted products, such as enterotoxins, exotoxins, and tissue damaging enzymes. The toxins kill or misguide immune cells which are important in the host defen

Host defence against S. aureus relies mainly on innate immunological mechanisms. The skin and mucous membranes are formidable barriers against invasion by Staphylococci. However,
adaptive response comes from the humoral arm of the immune system, and is mediated through three major mechanisms: promotion of opsonization, toxin neutralisation, and inhibition of
endothelial cells, and be internalised by a phagocytosislike process. Antibodies bound to specific antigens on the cell surface of bacteria serve as ligands for the attachment to PMNs and p

There is little clinical evidence that cell mediated immunity has a significant contribution in the defence against Staphylococci, yet one has to admit that the question is not adequately add
role in toxic shock syndrome and food poisoning, yet their function in routine infections is not well understood. Moreover, one cannot expect a long lasting antibody (memory) response w

',')
For all these above mentioned reasons, a tactic change on the war field against staphylococcal infections is badly needed. One way of combating infections is preventing them by active im
vaccination-directed towards surface components could both prevent bacterial adherence, neutralize toxins and promote phagocytosis. A vaccine based on fibronectin binding protein indu
IgG3 for opsonization, and any IgG subtype and IgA for neutralisation of adherence and toxin action. A chemically defined vaccine must be definitely superior compared to a whole cell v
dangerous side-effects.

Neonatal staphylococcal infections, severe septicemia and other life-threatening acute conditions are the primary target of passive immunisation. An effective vaccine offers great potentia

For the illustrative example concerning Staphylococcus aureus three different approaches have been employed in parallel. All three of these methods are based on the interaction of Staphy
selected sera.

- n
Following the high throughput screening, the selected antigenic proteins are expressed as recombinant proteins or in vitro translated products (in case it can not be expressed in prokaryoti
inhibit adhesion and promote phagocytosis. The antibodies against the secreted proteins are beneficial in toxin neutralisation. It is also known that bacteria communicate with each other th

The experimental approach includes the isolation of antibodies with the corresponding epitopes and proteins from human serum, and use them as reagents in the following assays: cell surf

The recognition of linear epitopes by antibodies can be based on sequences as short as 4-5 aa. Of course it does not necessarily mean that these short peptides are capable of inducing the g

The antibodies produced against Staphylococci by the human immune system and present in human sera are indicative of the in vivo expression of the-antigenic proteins and their immun

Accordingly, novel hyperimmune serum-reactive antigens from
Staphylococcus aureus or Staphylococcus epidermidis have been made available by the method according to the present invention.

According to another aspect of the present invention the invention relates to a hyperimmune serum-reactive antigen selected from the group consisting of the sequences listed in any one o
Tables 2a, 2b, 2c, 2d, 3,4 and 5, especially selected from the group consisting of Seq. ID No. 56,57,59,60,67,70,72,73, 74,75,76,77,78,79,80,81,82,85,87,88,89,90,92,95, 96,97,99,100,101
the group consisting of Seq. ID No. 56,57, 59,60,67,70,72,73,74,75,76, 77,78,79,80,81,82,85, 87,88,89,90,92,95,96,97,99,100,101,102,103,104,106, 108,110,112,114,116,118,120,122,12

Antigens from Staphylococcus aureus and Staphylococcus epidermidis have been extracted by the method according to the present invention which may be used in the manufacture of a p
Staphylococcus aureus and Staphylococcus epidermidis to be used in a pharmaceutical preparation are selected from the group consisting of the sequences listed in any one of Tables 2a, 2
Seq. ID No. 55,56,57,58,59,60,62,66,67,70,71,72,73, 74,75,76,77,78,79,80,81,82,83,84,85,87,88,89,90, 92,94,95,96,97,99,100,101,102,103,104,106,108,110, 112,114,116,118,120,122,1

A hyperimmune fragment is defined as a fragment of the identified antigen which is for itself antigenic or may be made antigenic when provided as a hapten. Therefore, also antigen or an
sera. preferred examples of such hyperimmune fragments of a hyperimmune serum-reactive antigen are selected from the group consisting of peptides comprising the amino acid sequenc
No. 55, aa 5-39,111-117,125-132,134-141,167-191,196-202,214-232, 236-241,244-249,292-297,319-328,336-341,365-380,385-391, 407-416,420-429,435-441,452-461,477-488,491-498

57, aa 33-43,45-51,57-63,65-72,80-96,99-110,123-129,161-171, 173-179,185-191,193-200,208-224,227-246,252-258,294-308, 321-329,344-352,691-707,358-411 and 588-606, of Seq. I
367,393-407,441-447,481-488,493-505,510-515,517-527,530535,540-549,564-583,593-599,608-621,636-645,656-670,674687,697-708,726-734,755-760,765-772,785-792,798-815,8198
1839-1851,1859-1866,1876-1882,1930-1939,1947-1954,1978-1985, 1999-2007,2015-2029,2080-2086,2094-2100,2112-2118,2196-2205, 2232-2243,198-258,646-727 and 2104-2206, of

62, aa 14-22,32-40,52-58,61-77,81-93,111-117,124-138, 151-190, 193-214,224-244,253-277,287-295,307-324,326-332,348-355, 357-362,384-394,397-434,437-460,489-496,503-510,51
No. 66, aa 49-56,62-68,83-89,92-98, 100,000 for some antigens, see Example 5) which are stable for > 1 year (see Example 1), suggests the existence of T-cell dependent memory most p
helperepitopes to. induce memory to T-independent antigens like for instance carbohydrates (conjugate vaccines). On the other hand, intracellular occurring staphylococci can be eliminat

T-cell epitopes can be predicted by various public domain algorithms:http ://bimas. dcrt. nih.gov/molbio/hla bind/ (Parker et al. 1994),http ://134.2.96.221/scripts/MHCServer. dll/home. h
ORFs corresponding to Seq ID 64 (IsaA), Seq ID 114 (POV2), SeqID 89 (ORF0222), Seq ID 70 (LPXTGIV), Seq ID 56 (LPXTGV), Seq
ID 142 (LPXTGVI), Seq ID 81(ORF3200), Seq ID 74 (ORF1951), Seq
ID 94 (Empbp), Seq ID 83 (autolysin) and Seq ID 58 (ORF2498) were analyzed using the TEPITOPE packagehttp ://my-page. ihost. com/usinet. hamme76/ (Sturniolo et al. 1999). The ar

The following peptides containing one or several promiscuous helper-epitopes were selected (and are claimed):
Seq ID 56 : pos. 6-40,583-598,620-646,871-896
Seq ID 58: no peptide fulfills selection criteria
Seq ID 64: no peptide fulfills selection criteria
Seq ID 70 : pos. 24-53
Seq ID 74 : pos. 240-260
Seq ID 81 : pos. 1660-1682,1746-1790
Seq ID 83 : pos. 1-29,680-709,878-902
Seq ID 89: pos. 96-136
Seq ID 94: pos. 1-29,226-269,275-326
Seq ID 114: pos. 23-47,107-156
Seq ID 142: pos. 24-53
The corresponding peptides or fragments thereof (for instance overlapping 15-mers) can be synthesized and tested for their ability to bind to various HLA molecules in vitro. Their immun
T-cells in vitro (Mayer et al. 1996, Schmittel et al. 2000, Ses ter et al.2000). In this regard it will be interesting to determine quantitative and qualitative differences in the T-cell response t

Moreover, a correlation between the antibody titers and the quan tity and quality of the T-cell response observed in these popula tions is expected. Alternatively, immunogenicity of the pr

1999).

Similar approaches can be taken for the identification of HLA class I restricted epitopes within staphylococcal antigens.

Synthetic peptides representing one or more promiscuous T helper epitopes from S. aureus
Partially overlapping peptides spanning the indicated regions of
Seq ID 56 (LPXTGV), Seq ID 70 (LPXTGIV), Seq ID 74(ORFlhoml),
Seq ID 81(EMBP), Seq ID 83 (Autolysin), Seq ID 89 (ORFlhom2),
Seq ID 94 (EMPBP), Seq ID 114 (POV2) and Seq ID 142 (LPXTGVI) were synthesized. Sequences of the individual peptides are given in Table 5. All peptides were synthesized using F
HPLC purified and analyzed by mass spectrometry. Lyophilized pep tides were dissolved in DMSO and storedat-20 C at a concentra tion of 5-10 mM.

Binding of synthetic peptides representing promiscuous T helper epitopes to HLA molecules in vitro
Binding of peptides to HLA molecules on the surface of antigenpresenting cells is a prerequisite for activation of T cells.

Binding was assessed in vitro by two independent biochemical assays using recombinant soluble versions of HLA class II molecules. One assay measures the concentration dependent cor

The second assay is based on the formation ofSDS-stable complexes upon binding of high-and intermediate affinity ligands.

A summary of the results obtained by the two assays is given in
Table 5.

Soluble HLA molecules(DRA1*0101/DRB1*0101 andDRA1*0101/DRB1*0401) were expressed in SC-2 cells and purified as described in Aichinger et al., 1997. For the competition ass
DRB1*0401 biotinilated indicator peptide UD4(YPKFVKQNTLKAA,
Valli et al. 1993) was used between 0.03 and 0.06AM. Test peptides were used in serial dilutions from 0.02 nM to 200AM. Molecules, indicator and test peptides were incubated overnigh
using a streptavidin-alkaline phosphatase conjugate (Dako) with NBT/BCIP tablets (Sigma) as substrate and automated OD reading on a Victor reader (Wallac).

*T cell response against promiscuous T helper epitopes assessed byIFNg ELIspot assay
Upon antigenic stimulation T cells start to proliferate and to secrete cytokines such as interferon gamma (IFNg). Human T cells specifically recognizing epitopes within S. aureus antigens
(Amersham) and PPD (tuberculin purified protein derivate, Statens Serum Institute) served as assay positive controls, assay medium without any peptide as negative control. After overnig
(Bioreader 2000, BIO-SYS). Spots counted in wells with cells stimulated with assay medium only (negative control, generally below 10 spots/100.000 cells) were regarded as background

Table 5: Promiscuous T helper epitopes contained in S. aureus antigens
EMI52.1

<tb> Amino <SEP> acid <SEP> sequences <SEP> within <SEP> S. <SEP> aureus <SEP> antigens <SEP> containing <SEP> binding <SEP> IFNg
<tb> highly <SEP> promiscuous <SEP> T <SEP> helper <SEP> epitopes <SEP> ELIspot
<tb> <SEP> 2)
<tb> Seq <SEP> ID <SEP> 56 <SEP> (LPXTGV): <SEP> pos. <SEP> 6-40
<tb> p6-28 <SEP> > PKLRSFYSIRKSTLGVASVIVST//+
<tb> p24-40 <SEP> > VIVSTLFLISQHQAQA//
<tb> <SEP> 44; <SEP> 80; <SEP> 8
<tb> <SEP> ; <SEP> 95 <SEP> ; <SEP> 112
<tb> Seq <SEP> ID <SEP> 56 <SEP> (LPXTGV) <SEP> : <SEP> pos. <SEP> 620-646
<tb> p620-646 <SEP> > FPYIPDKAVYNAIVKWVANIGYEGQ//+
<tb> Seq <SEP> ID <SEP> 56 <SEP> (LPXTGV): <SEP> pos. <SEP> 871-896
<tb> p871-896 <SEP> > QSWWGLYALLGMLALFIPKFRKESK//
<tb> Seq <SEP> ID <SEP> 70 <SEP> (LPXTGIV): <SEP> pos. <SEP> 24-53
<tb> p24-53 <SEP> > YSIRKFTVGTASILIGSLMYLGTQQEAEA//nd <SEP> 34; <SEP> 14; <SEP> 0
<tb> <SEP> ; <SEP> 57 <SEP> ; <SEP> 16
<tb> Seq <SEP> ID <SEP> 74 <SEP> (ORFlhoml) <SEP> : <SEP> pos. <SEP> 240-260
<tb> p240-260 <SEP> > MNYGYGPGVVTSRTISASQA//+ <SEP> 47 <SEP> ; <SEP> 50; <SEP> 0
<tb> <SEP> ; <SEP> 85 <SEP> ; <SEP> 92
<tb>
EMI53.1

<tb> Seq <SEP> ID <SEP> 81 <SEP> (EM~BP) <SEP> : <SEP> pos. <SEP> 1660-1682
<tb> pl660-1682 <SEP> > NEIVLETIRDINNAHTLQQVEA//nd
<tb> <SEP> 2; <SEP> 14; <SEP> 5;
<tb> <SEP> 77 <SEP> ; <SEP> 26
<tb> Seq <SEP> ID <SEP> 81 <SEP> (EM~BP) <SEP> : <SEP> pos. <SEP> 1746-1790
<tb> pl746-1773 <SEP> > LHMRHFSNNFGNVIKNAIGWGISGLLA//nd
<tb> pl753-1779 <SEP> > NNFGNVIKNAIGWGISGLLASFWFFI//nd
<tb> p1777-1789 <SEP> > FFIAKRRRKEDEE/ <SEP> nd
<tb> Seq <SEP> ID <SEP> 83 <SEP> (Autolysin) <SEP> pos. <SEP> 1-29
<tb> pl-29 <SEP> : <SEP> > MAKKFNYKLPSMVALTLVGSAVTAHQVQA//nd
<tb> <SEP> 6; <SEP> 35; <SEP> 7;
<tb> <SEP> 60 <SEP> ; <SEP> 49
<tb> Seq <SEP> ID <SEP> 83 <SEP> (Autolysin) <SEP> pos. <SEP> 878-902
<tb> p878-902: <SEP> > NGLSMVPWGTKNQVILTGNNIAQG/nd
<tb> Seq <SEP> ID <SEP> 89 <SEP> (ORFlhom2): <SEP> pos. <SEP> 96-136
<tb> p96-121 <SEP> > GESLNIIASRYGVSVDQLMAANNLRG//
<tb> pll7-136 <SEP> > NNLRGYLIMPNQTLQIPNG//-0 <SEP> ; <SEP> 35; <SEP> 0;
<tb> <SEP> 29 <SEP> ; <SEP> 104
<tb> Seq <SEP> ID <SEP> 94 <SEP> (EMPBP): <SEP> pos. <SEP> 1-29
<tb> p4-29: <SEP> > KLLVLTMSTLFATQIMNSNHAKASV//+
<tb> Seq <SEP> ID <SEP> 94 <SEP> (EMPBP): <SEP> pos. <SEP> 226-269
<tb> p226-251 <SEP> > IKINHFCWPQINSFKVIPPYGHNS//
<tb> p254-270 <SEP> > MHVPSFQNNTTATHQN//+
<tb> <SEP> 26 <SEP> ; <SEP> 28; <SEP> 1
<tb> <SEP> 6; <SEP> 43; <SEP> 97
<tb> Seq <SEP> ID <SEP> 94 <SEP> (EMPBP): <SEP> pos. <SEP> 275-326
<tb> p275-299 <SEP> > YDYKYFYSYKVVKGVKKYFSFSQS//+
<tb> p284-305 <SEP> > YKWKGVKKYFSFSQSNGYKIG//+
<tb> p306-326 <SEP> > PSLNIKNVNYQYAVPSYSPT//
<tb> Seq <SEP> ID <SEP> 114 <SEP> (POV2): <SEP> pos. <SEP> 23-47
<tb> p23-47 <SEP> > AGGIFYNQTNQQLLVLCDGMGGHK//-49 <SEP> ; <SEP> 20; <SEP> 4
<tb> <SEP> ; <SEP> 77; <SEP> 25
<tb> Seq <SEP> ID <SEP> 114 <SEP> (POV2): <SEP> pos. <SEP> 107-156
<tb> p107-124 <SEP> > ALVFEKSWIANVGDSRA/
<tb> pl26-146 <SEP> > RAYVINSRQIEQITSDHSFVN//nd
<tb> pl42-158 <SEP> > SFVNHLVLTGQITPEE//nd
<tb> Seq <SEP> ID <SEP> 142 <SEP> (LPXTGVI): <SEP> pos. <SEP> 1-42
<tb> p6-30 <SEP> > KEFKSFYSIRKSSLGVASVAISTL//++
<tb> pl8-42 <SEP> > SSLGVASVAISTLLLLMSNGEAQA//nd
<tb> <SEP> 0; <SEP> 41; <SEP> 20
<tb> <SEP> ;88;109
<tb> Seq <SEP> ID <SEP> 142 <SEP> (LPXTGVI): <SEP> pos. <SEP> 209-244
<tb> p209-233 <SEP> > IKLVSYDTVKDYAYIRFSVSNGTKA//+
<tb> p218-244 <SEP> > KDYAYIRFSVSNGTKAVKIVSSTHFNN//+
<tb> Seq <SEP> ID <SEP> 142 <SEP> (LPXTGVI): <SEP> pos. <SEP> 395-428
<tb> p395-418 <SEP> > FMVEGQRVRTISTYAINNTRCTIF//
<tb> p416-428 <SEP> > TIFRYVEGKSLYE//
<tb>
EMI54.1

<tb> Seq <SEP> ID <SEP> 142 <SEP> (LPXTGVI): <SEP> pos. <SEP> 623-647
<tb> p623-647 <SEP> > MTLPLMALLALSSIVAFVLPRKRKN//~
<tb> 1) binding to solubleDRA1*0101/DRB1*0401 molecules was determined using a competition assay (+, ++: binding,- no competition up to 200 uM test peptide; nd: not done)2) resu

Data are represented as spots/200.000 cells (background values are subtracted 5. Antigens may be injected into mice-and the antibodies against these proteins can be measured.

6. Protective capacity of the antibodies induced by the antigens through vaccination can be assessed in animal models.

Both 5. and 6. are methods well available to the skilled man in the art.

Example 7: Applications i t A) An effective vaccine offers great potential for patients facing elective surgery in general, and those receiving endovascular devices, in particular. Patients st administration with all its dangerous side-effects.

Therefore an effective vaccine offers great potential for patients facing elective surgery in general, and those receiving endovascular devices, in particular.

S. aureus can cause many different diseases.

1. Sepsis, bacteriaemia 2. Haemodialysedpatients-bacteriemia, sepsis 3. Peritoneal dialyses patients-peritonitis 4. Patients with endovascular devices (heartsurgery, etc)-en- docarditis, bac
B) Passive and active vaccination, both with special attention to
T-cells with the latter one: It is an aim to induce a strong T helper response during vaccination to achieve efficient humoral response and also immunological memory. Up till now, there is
S. aureus infections, however, it was not adequately addressed, so far. An effective humoral response against proteinaceous antigens must involve T help, and is essential for developing m

Since, innate immunological responses (cytokines) will influence this decision, the involvement of T-cells might be different during an acute, serious infection relative to immunization of

C) Preventive and therapeutic vaccines
Preventive: active vaccination/passive immunization of people in high risk groups, before infection
Therapeutic: passive vaccination of the already sick.

Active vaccination to remove nasal carriage
Specific example for an application
Elimination of MRSA carriage and prevention of colonization of the medical staff
Carriage rates of S. aureus in the nares of people outside of the hospitals varies from 10 to40%. Hospital patients and personnel have higher carriage rates. The rates are especially high in

The ELISA data strongly suggest that there is a pronounced IgA response to S. aureus, which is not obvious or known from the literature. Since the predominant mucosal immune respons

Clear indication : Everybody's threat in the departments where they perform operation (esp. orthopedics, traumatology, gen. surgery)
Well-defined population for vaccination (doctors and nurses)
Health care workers identified as intranasal carriers of an epidemic strain of S. aureus are currently treated with mupi rocin and rifampicin until they eliminate the bacteria. Some times it i

Available animal model: There are mice models for intranasal carriage.

Table 1: ELISA titers of sera from non-infected individuals against multiple staphylococcal proteins.
EM157.1

era <SEP> ID# <SEP> HI <SEP> TA <SEP> G <SEP> 1fA <SEP> 1+D3 <SEP> nBPA <SEP> drE <SEP> drC <SEP> BP <SEP> nolase <SEP> P309 <SEP> P342 <SEP> oagul
<tb> lysate
<tb> 1
<tb> 2..... <SEP> 3.....
<tb>

3 <SEP> 7..... <SEP> 1..... <SEP> 2..... <SEP> 7..... <SEP> 4.....
<tb>

4 <SEP> 1****** <SEP> 1****** <SEP> 1..... <SEP> 6..... <SEP> 2..... <SEP> 6..... <SEP> 2..... <SEP> 6, <SEP> 7..... <SEP> 1..... <SEP> 3.....
<tb>

5
<tb> a
<tb> i.....
<tb>

:., <SEP> 9.....
<tb>

9 <SEP> 5, <SEP> 1....., <SEP> 4..... <SEP> 1....., <SEP> 6.....
<tb>

2 <SEP> 2 <SEP> 2, <SEP> B <SEP> 4 <SEP> ~ <SEP> S <SEP> 3
<tb> 3 <SEP> 7. <SEP> 3 <SEP> 1 <SEP> 1. <SEP> 3 <SEP> 2 <SEP> 2 <SEP> 2 <SEP> 3 <SEP> 7 <SEP> 4
<tb> 4 <SEP> 1****** <SEP> 1****** <SEP> 1 <SEP> 6 <SEP> 2 <SEP> 2 <SEP> 3 <SEP> 5 <SEP> 2 <SEP> 6, <SEP> 7 <SEP> : <SEP> 8 <SEP> 3 <SEP> ~ <SEP> T <SEP> 1 ·
<tb> S <SEP> ~.. <SEP> i,
<tb> i <SEP> 1....
<tb>

124, <SEP> 5.....
<tb>

13 <SEP> i...
<tb>

14
<tb> 11 <SEP> 6 <SEP> 45-6
<tb> 3.....
<tb>

14
<tb> L5 <SEP> 3 <SEP> 5 <SEP> 2, <SEP> 3 <SEP> 5 <SEP> 6 <SEP> 7 <SEP> 8 <SEP> 8, <SEP> 9
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17
<tb> 191.....
<tb>

19 <SEP> 11.....
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~ <SEP> ~~ <SEP> ~
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EMI58.1

I <SEP> H <SEP> N <SEP> I <SEP> G <SEP> Cl <SEP> n <SEP> X <SEP> tBP <SEP> tnol <SEP> sc <SEP> tP309 <SEP> tP3x
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<tb> 8, <SEP> 9.....
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25 <SEP> 5.....
<tb>

26..... <SEP> 7.....
<tb>

28', <SEP> J
<tb> 8
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2.....
<tb>

3
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354, <SEP> 5, <SEP> 6.... <SEP> 8..... <SEP> 2, <SEP> 3..... <SEP> 5..... <SEP> 1******3..... <SEP> 4.....
<tb>

5,35.
<tb>

.., <SEP> 8.... <SEP> :..
<tb>

,...
<tb>

38 <SEP> 8..... <SEP> 3, <SEP> 4.....
<tb>

39J
<tb> 0....., <SEP> ; <SEP> , <SEP> 5...., <SEP> 9.....
<tb>

Table1. ELISA titers of sera from non-infected individuals against multiple staphylococcal proteins.

Anti-staphylococcal antibody levels were measured individually by standard ELISA with total lysate prepared from S. aureus grown in
BHI medium (BHI), lipoteichoic acid (LTA), peptidoglycan (PG), 13 recombinant proteins, representing cell surface and secreted proteins, such as clumping factor A and B (ClfA, ClfB),
Class II analogous protein (map-w), Elastin-binding protein (EBP), enolase (reported to be cell surface located and immunogenic), iron transport lipoproteins (LP309, LP342), sortase (srt
IgG titer, and obtained a score from 1-9. Score 1 labels the highest titer serum and score 8 or 9 labels the sera which were 8th or9th among all the sera tested for the given antigen. It result

Thefive"best sera"meaning the most hyper reactive in terms of anti-staphylococcal antibodies were selected based on the number of scores 1-8. **** means that the antibody reactivity ag

Table 2a: Immunogenic proteins identified by bacterial surface and ribosome display: S. aureus
Bacterial surface display: A, LSA250/1 library in fhuA with patient sera 1 (655); B, LSA50/6 library in lamB with patient sera 1(484) ; C, LSA250/1 library in fhuA with IC sera 1 (571);
LSA50/6 library in lamB with IC sera 2 (454); F, LSA50/6 library in lamB with patient sera PI (1105); G, LSA50/6 library in lamb with IC sera 1 (471)) ; H, LSA250/1 library in fhuA wi
GENIC (Kolaskar and Tongaonkar, 1990); #, identical sequence present twice in ORF; ##, clone not in database (not sequence by
TIGR).
EMI60.1

<tb>

<SEP> S. <SEP> Old <SEP> Putative <SEP> function <SEP> predicted <SEP> immunogenic <SEP> aa** <SEP> No. <SEP> of <SEP> sc-Location <SEP> of <SEP> Serum <SEP> re
<tb> <SEP> aureus <SEP> ORF <SEP> (by <SEP> homology) <SEP> lected <SEP> identified <SEP> with <SEP> relevant <SEP> re- <SEP> (DNA
<tb> antigenic <SEP> number <SEP> clones <SEP> pc <SEP> immuno-gion <SEP> (positive/total <SEP> ¦ <SEP> +Prot)
<tb> <SEP> protein <SEP> ORF <SEP> and <SEP> genic <SEP> region
<tb> <SEP> screen
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<tb> <SEP> 189) <SEP> : <SEP> 26/30
<tb> SaA0003 <SEP> ORF2967P <SEP> repC <SEP> 7-19,46-57,85-91, <SEP> 110-117, <SEP> 125- <SEP> C <SEP> : <SEP> 18 <SEP> aa <SEP> 9-42 <SEP> C: <SEP> GSBY15
<tb> <SEP> 133,140-149,156-163,198-204, <SEP> aa <SEP> 158-174 <SEP> 42): <SEP> 1/1
<tb> <SEP> 236-251,269-275,283-290,318
<tb> <SEP> 323,347-363

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<tb> <SEP> 157,173-180,186-205,215-226, <SEP> C: <SEP> 1, <SEP> F: <SEP> 6, <SEP> aa <SEP> 684-764 <SEP> 182): <SEP> 9/30
<tb> <SEP> 239-263,269-274,284-304,317-G: <SEP> 2 <SEP> aa <SEP> 836-870 <SEP> D: <SEP> n. <SEP> d.
<tb>

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<tb> <SEP> 372-379,391-397,399-406, <SEP> 413- <SEP> 870) <SEP> : <SEP> 3/16
<tb> <SEP> 425,430-436,444-455,499-505,
<tb> <SEP> 520-529, <SEP> 553-568, <SEP> 586-592, <SEP> 600
<tb> <SEP> 617,631-639,664-678,695-701,
<tb> <SEP> 891-903,906-912,926-940
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<tb> <SEP> 217-223,232-238,246-261, <SEP> 266- <SEP> 192) <SEP> : <SEP> 2/14
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<tb> <SEP> 353-360,367-380,384-396, <SEP> 404- <SEP> 162) <SEP> : <SEP> 23/41
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<tb> <SEP> 181):25/41
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<tb> <SEP> ORFI <SEP> 59-73,90-96,99-113,160-173, <SEP> C: <SEP> 18*, <SEP> aa <SEP> 103-166 <SEP> 95) <SEP> : <SEP> 1/1
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<tb> <SEP> 262 <SEP> 166): <SEP> 14/29
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<tb> <SEP> 153) <SEP> : <SEP> 47/51
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<tb> <SEP> -431) <SEP> : <SEP> 10/27
EMI61.1

<tb> <SEP> S. <SEP> Old <SEP> Putative <SEP> function <SEP> predicted <SEP> immunogenic <SEP> aa** <SEP> No. <SEP> of <SEP> se-Location <SEP> of <SEP> Serum <SE
<tb> <SEP> aureus <SEP> ORF <SEP> (by <SEP> homology) <SEP> lected <SEP> identified <SEP> with <SEP> relevant <SEP> re- <SEP> (DNA
<tb> antigenic <SEP> number <SEP> clones <SEP> pe <SEP> immuno-gion <SEP> (positive/total) <SEP> +Prot)
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<tb> <SEP> 177): <SEP> 13/41
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EM170.1

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Table 2b: Additional immunogenic proteins identified by bacterial surface and ribosome display: S. aureus
Bacterial surface display: A, LSA250/1 library infhuA with patient sera 1 (655); B, LSA50/6 library in lamB with patient sera 1 (484); C, LSA250/1 library infhuA with IC sera 1 (571); E LSA50/6 library in lamB with IC sera 2 (454); F, LSA50/6 library in lamB with patient sera Pi (1105); G, LSA50/6 library in lamb with IC sera 1 (471); H, LSA250/1 library infhuA with EMI71.1

<tb>

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<tb> 0
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EMI72.1

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<tb> <SEP> 409-416
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EMI78.1

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<tb> c <SEP> protein <SEP> per <SEP> ORF <SEP> genic <SEP> region <SEP> +Prot)
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<tb> <SEP> 273-299,301-326,328-355,366
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<tb> <SEP> 221-227,232-237
<tb> ORF290 <SEP> Conserved <SEP> hypo-4-27,34-43,62-73,81-90,103-116, <SEP> D: <SEP> 24 <SEP> aa <SEP> 360-376 <SEP> D: <SEP> n.d. <SEP> 324,352
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<tb> 8 <SEP> 91-107, <SEP> 129-141, <SEP> 149-155, <SEP> 165-178,
<tb> <SEP> 188-194
<tb> ORF297 <SEP> Surface <SEP> protein, <SEP> 9-23,38-43,55-60,69-78,93-101, <SEP> H <SEP> : <SEP> 5 <SEP> aa <SEP> 1-70H <SEP> : <SEP> GSBYU66: <SEP> n. <SEP
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<tb> <SEP> 410, <SEP> 419-426,463-475,482-491
<tb>
Table 2c : Immunogenic proteins identified by bacterial surface and ribosome display : S. epidermidis.

Bacterial surface display: A, LSE150 library infhuA with patient sera 2(957) ;B, LSE70 library in lamB with patient sera 2(1420) ; C, LSE70 library in lamB with patient sera1 (551). Rib
CRF, reading frame on complementary strand. ORF, open reading frame; CRF, reading frame on complementary strand.
EMI79.1

<tb>

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<tb> epidermidi <SEP> (by <SEP> homology) <SEP> selected <SEP> identified <SEP> region <SEP> (positive/total) <SEP> no:
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<tb> <SEP> protein <SEP> per <SEP> ORF <SEP> genic <SEP> region <SEP> +Prot)
<tb> <SEP> and
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<tb> ARF0172 <SEP> cation-transport-4-34, <SEP> 37-43 <SEP> D: <SEP> 6 <SEP> aa3-32 <SEP> D: <SEP> nd <SEP> 497,
<tb> <SEP> ing <SEP> ATPase, <SEP> El-548
<tb> <SEP> E2 <SEP> family
<tb> ARF0183 <SEP> condensing <SEP> en-4-22,24-49 <SEP> D: <SEP> 4 <SEP> aal-52 <SEP> D: <SEP> nd <SEP> 498,
<tb> <SEP> zyme, <SEP> putative, <SEP> 549
<tb> <SEP> FabH-related
<tb> ARF2455 <SEP> NADH <SEP> 4-29 <SEP> D: <SEP> 3 <SEP> aal-22 <SEP> D: <SEP> nd <SEP> 499,
<tb> <SEP> dehydrogenase, <SEP> 550
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<tb> <SEP> 551
<tb> CRF0002 <SEP> Unknown <SEP> 4-13,15-23,36-62 <SEP> D: <SEP> 5 <SEP> aa21-70 <SEP> D: <SEP> nd <SEP> 501,
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<tb> <SEP> 554
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<tb> <SEP> 559
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<tb> <SEP> 448

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Table 2d: Immunogenic proteins identified by bacterial surface and ribosome display: S. aureus (new annotation)
Bacterial surface display: A, LSA250/1 library infhuA with patient. sera 1 (655); B, LSA50/6 library in lamB with patient sera 1 (484); C, LSA250/1 library infhuA with IC sera 1 (571);E
LSA50/6 library in lamB with IC sera 2 (454);F, LSA50/6 library in lamB with patient sera P1 (1105);G, LSA50/6 library in lamb with IC sera 1 (471). Ribosome display:D, LSA250/1 lil
ORF.
EMI91.1

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EMI93.1

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<tb> <SEP> terminus <SEP> 290-296,298-305,314-319,327-C: <SEP> GSBYN80 <SEP> (98-190): <SEP> 13/17
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<tb> ORF2963 <SEP> ORF2295 <SEP> putative <SEP> 8-23,35-41,64-70,81-87,109-115, <SEP> C: <SEP> 3, <SEP> E: <SEP> 3, <SEP> aa <SEP> 17-95 <SEP> C: <SEP> GSBYJ5
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<tb> <SEP> terminus <SEP> 265-271, <SEP> 279-285
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EMI94.1

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<tb> <SEP> protein <SEP> ORF <SEP> and <SEP> genic <SEP> re-+Prot)
<tb> <SEP> screen <SEP> gion
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EMI95.1

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4894-4909,4937-4942,5047-5054,
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5226-5236,5278-5284, 5315-5321,
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7974-7981, 79998005,8039-8045,8049-8065,80708075,8099-8112,8119-8125,81518158,8169-8181,8226-8232,82588264,8291-8299,8301-8310,83258335,8375-8389,8394-8400,840584
pool <SEP> N22 <SEP> 1 <SEP> : <SEP> 10.000
<tb> <SEP> 1: <SEP> 20,000 <SEP> 1: <SEP> 50,000 <SEP> each <SEP> C2, <SEP> 5,6,10,12 <SEP> IC40 <SEP> 1 <SEP> : <SEP> 50,000
<tb> <SEP> 1: <SEP> 10,000
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<tb> PCK4 <SEP> + <SEP> +++ <SEP> +++
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<tb> PCK6 <SEP> + <SEP> + <SEP> - <SEP> +
<tb>
EMI97.2

<tb> <SEP> Spot <SEP> ID/sera <SEP> IC35, <SEP> 40 <SEP> P-pool <SEP> Infant <SEP> pool
<tb> <SEP> 1: <SEP> 50,000 <SEP> (P6,18,25,28,29) <SEP> C2,5,6,10,12
<tb> <SEP> N22 <SEP> 1:10,000 <SEP> 1:50,000 <SEP> each <SEP> 1:10,000
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<tb> PAC3
<tb> PAC5 <SEP> ++
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EMI97.3

<tb> <SEP> Spot <SEP> ID/sera <SEP> P-pool <SEP> Infant <SEP> 14 <SEP> IC <SEP> pool/IgG <SEP> IC <SEP> pool/IgA
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<tb> PAC11 <SEP> ++ <SEP> ++ <SEP> ++
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<tb>
EMI98.1

P <SEP> OV38 <SEP> ++
<tb> P <SEP> OV39 <SEP> +++
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<tb> b) S. aureus/COL"standard conditions"
EMI98.2

<tb> <SEP> Spot <SEP> ID/sera <SEP> IC <SEP> pool <SEP> IC35 <SEP> P18 <SEP> P25 <SEP> P1 <SEP> P29 <SEP> Infant <SEP> 18
<tb> <SEP> (N26, <SEP> IC34, <SEP> 35) <SEP> 1: <SEP> 20,000 <SEP> 1: <SEP> 10, <SEP> 000 <SEP> 1 <SEP> : <SEP> 10,000 <SEP> 1: <SEP> 5,000 <SEP> 1: <SEP> 2,500
<tb> <SEP> 1: <SEP> 30,000 <SEP> each
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<tb> POV3.1 <SEP> +++ <SEP> +++ <SEP> +++ <SEP> +++ <SEP> +++
<tb> POV3.2 <SEP> +++ <SEP> +++ <SEP> +++ <SEP> +++ <SEP> +++
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<tb> POV7 <SEP> - <SEP> - <SEP> +++ <SEP> - <SEP> - <SEP>
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<tb> POV12 <SEP> - <SEP> - <SEP> - <SEP> - <SEP> - <SEP> +++
<tb> POV13 <SEP> ++ <SEP> +++ <SEP> +++ <SEP> +++ <SEP> ++ <SEP> ++
<tb> POV14 <SEP> ++ <SEP> +++ <SEP> +++ <SEP> ++ <SEP> ++ <SEP> ++
<tb> POV15 <SEP> + <SEP> + <SEP> - <SEP> + <SEP> (+)
<tb> c) S. aureus/COL"stress conditions"
EMI98.3

<SEP> Spot <SEP> ID/sera <SEP> P-pool <SEP> IC34+IC35 <SEP> P18 <SEP> P29 <SEP> Infant <SEP> 14
<tb> <SEP> (P6,18,25,28,29) <SEP> 1: <SEP> 20,000 <SEP> each <SEP> 1: <SEP> 10,000 <SEP> 1: <SEP> 10,000 <SEP> 1: <SEP> 10,000
<tb> <SEP> 1 <SEP> : <SEP> 50,000 <SEP> each
<tb> POV16 <SEP> +++
<tb> POV17
<tb> POV18 <SEP> + <SEP> - <SEP> ++
<tb> POV19
<tb> POV21 <SEP> - <SEP> - <SEP> +
<tb> POV23 <SEP> - <SEP> + <SEP>
<tb> POV24 <SEP> - <SEP> + <SEP>
<tb> POV25 <SEP> + <SEP> - <SEP>
Table 4. S. aureus antigens identified byMALDI-TOF-MS sequencing (ORFs in bold were also identified by bacterial surface display)
Prediction of antigenic regions in selected antigens identified by serological proteome analysis using human sera
EMI99.1

<tb> <SEP> spot <SEP> ID <SEP> S. <SEP> aureus <SEP> pro-Putative <SEP> function <SEP> (by <SEP> homology) <SEP> Seq <SEP> ID <SEP> no <SEP> : <SEP> Putative <SE
<tb> <SEP> tein <SEP> (DNA, <SEP> Prot) <SEP> ization
<tb> <SEP> (ORF <SEP> no./ab
<tb> <SEP> brev.)
<tb> PCK2 <SEP> ORF0599 <SEP> Glycinamide-ribosyl <SEP> synthase <SEP> 107,108 <SEP> cytoplasmic
<tb> PCK5 <SEP> ORF0484 <SEP> yitU <SEP> conserved <SEP> hypoth. <SEP> protein <SEP> (yitU) <SEP> 109, <SEP> 110 <SEP> cytoplasmic
<tb> PCK6 <SEP> ORF2309 <SEP> membrane-associated <SEP> malate-quinone <SEP> 111,112 <SEP> peripheral <SEP> mem
<tb> <SEP> mqo <SEP> oxidase <SEP> branc
<tb> POV2 <SEP> ORF0766 <SEP> aux1 <SEP> protein <SEP> phosphatase <SEP> contributing <SEP> to <SEP> me-113,114 <SEP> trans-membrane
<tb> <SEP> thicilin <SEP> resistance
<tb> POV4, <SEP> 17 <SEP> ORF0078 <SEP> EF-C-terminal <SEP> part <SEP> of <SEP> 44 <SEP> kDa <SEP> protein <SEP> similar <SEP> 115,116 <SEP> cytoplasmic/se
<tb> PAC14,19 <SEP> Tu <SEP> to <SEP> elongation <SEP> factor <SEP> Tu <SEP> creted
<tb> POV5 <SEP> ORF0782 <SEP> 3-ketoacyl-acyl <SEP> carrier <SEP> protein <SEP> reduc-117,118 <SEP> cytoplasmic
<tb> <SEP> tase <SEP> (fabG)
<tb> POV7 <SEP> ORF0317 <SEP> SecA <SEP> protein <SEP> transport <SEP> across <SEP> the <SEP> membrane <SEP> 39,91 <SEP> cytoplasmic
<tb> <SEP> SecA
<tb> POV10 <SEP> ORF1252 <SEP> yrzC <SEP> hypothetical <SEP> BACSU <SEP> 11.9 <SEP> kd <SEP> protein <SEP> 119,120 <SEP> cytoplasmic
<tb> <SEP> (up0074 <SEP> (rff2) <SEP> family)
<tb> POV12 <SEP> ORF0621 <SEP> pdhB <SEP> dihydrolipoamide <SEP> acetyltransferase <SEP> 121,122 <SEP> cytoplasmic
<tb> <SEP> (pdhB)
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<tb> POV15 <SEP> ORF0077 <SEP> EF-85 <SEP> kD <SEP> vitronectin <SEP> binding <SEP> protein <SEP> 127,128 <SEP> cytoplasmic
<tb> <SEP> G
<tb> POV18 <SEP> not <SEP> found <SEP> general <SEP> stress <SEP> protein <SEP> YLY1 <SEP> 129,130 <SEP> cytoplasmic
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<tb> <SEP> gidA
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<tb> <SEP> prsA <SEP> (prsA)
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<tb> <SEP> (ModA <SEP> (ModA)
<tb> PAC3 <SEP> ORF2053 <SEP> heavy <SEP> metal <SEP> dependent <SEP> transcriptional <SEP> 177,178 <SEP> cytoplasmic
<tb> <SEP> activator, <SEP> putative <SEP> regulator <SEP> of <SEP> multidrug

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<tb> <SEP> ydaP
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<tb> <SEP> alaS
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<tb> <SEP> ymfA
<tb> PAC15 <SEP> ORF1124 <SEP> lipoamid <SEP> acyltransferase <SEP> component <SEP> of <SEP> 163,164 <SEP> cytoplasmic
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<tb> <SEP> similar <SEP> to <SEP> hypothetical <SEP> proteins
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<tb> <SEP> tein <SEP> (DNA, <SEP> Prot) <SEP> ization
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EMI101.2

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<tb> <SEP> (Protein) <SEP> no./abbrev. <SEP> ization <SEP> (Antigenic <SEP> package)
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<tb> <SEP> branc <SEP> 136-149,175-183,185-193,206-212,222
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<tb> 116 <SEP> POV4 <SEP> ORF078 <SEP> EF-Tu <SEP> cytoplasmic/28-38, <SEP> 76-91, <SEP> 102-109,118-141,146-153,
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<tb> 174 <SEP> PAC1 <SEP> ORF1490 <SEP> periplasmic <SEP> 5-24,38-44,100-106, <SEP> 118-130,144-154,
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<tb> PCK5 <SEP> TIGR6209 <SEP> ORF0484 <SEP> yitU <SEP> conserved <SEP> hypoth. <SEP> protein <SEP> (yitU) <SEP> 109,110
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<tb> <SEP> oxidase
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<tb> POV13 <SEP> 7470965 <SEP> ORF0094 <SEP> SdrD <SEP> fibrinogen-bdg. <SEP> (LPXTG) <SEP> protein <SEP> homolog <SEP> 123,124
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<tb> ') identified from a total lysate from S. aureus 8325-4 spa-grown under standard conditions. Seroreactivity with 1/1 patient and 2/4 normal sera but not with infant serum (C5).

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Claims of **WO02059148**

Claim : 1. Method for identification, isolation and production of hyperimmune serum-reactive antigens from a pathogen, a tumor, an allergen or a tissue or host prone to auto-immunity, s individual sera with antibodies against said specific pathogen, tumor, al lergen or tissue or host prone to auto-immunity, providing at least one expression library of said specific pathogen, identified antigens with individual antibody preparations from individual sera from individuals with anti bodies against said specific pathogen, tumor, allergen or tis sue or host prone to a and optionally isolating said hyperimmune serum-reactive antigens and producing said hyperimmune serum-reactive antigens by chemical or recombinant methods, 2. Method for identifi steps : providing an antibody preparation from a plasma pool of said given type of animal or from a human plasma pool or individual sera with antibodies against said specific pathogen, p antibodies in said antibody prepara tion, screening the identified antigens with individual antibody preparations from individual sera from individuals with anti bodies against said specific said screening and identification steps at least once, comparing the hyperimmune serum-reactive antigensidentified'~~ in the repeated screening and identification steps with the hyperimm and identification steps only, until less than 5% of the hyperimmune serum-reac tive antigens are identified in a further repeating step only to obtain a complete set of hyperimmune serum

3. Method according to claim 1 or 2 characterized in that at least one of said expression libraries is selected from a ribosomal display library, a bacterial surface library and a proteome.

4. Method according to'claim 2 characterized in that said at least three different expression libraries are at least a ribsomal display library, a bacterial surface library and a prteome.

5. Method according to any one of claims 1 to 4, characterized in that said plasma pool is a human plasma pool taken from individuals having experienced or are experiencing an infection

6. Method according to any one of claims 1 to 5, characterized in that said expression libraries are genomic expression libraries of said pathogen.

7. Method according to any one of claims 1 to 6, characterized in that said expression libraries are complete genomic expression libraries, preferably with a redundancy of at least 2x, mor

8. Method according to any one of claims 1 to 7, characterized in that it comprises the steps of screening at least a ribosomal display library, a bacterial surface display library and a protec

9. Method according to any one of claims 1 to 8, characterized in that said pathogen is selected from the group of bacterial, viral, fungal and protozoan pathogen.

10. Method according to any one of claims 1 to 9, characterized in that said pathogen is selected from the group of human immunedeficiency virus, hepatitis A virus, hepatitis B virus, hep
Streptococcus pneumoniae,
Streptococcuspyogenes, Streptococcus agalactiae, Enterococcus faecalis, Bacillusanthracis, Vibrio cholerae, Borrelia burgdorferi, Plasmodium sp., Aspergillus sp. or Candida albicans.

11. Method according to any one of claims 1 to 10, characterized in that at least one of said expression libraries is a ribosomal display library or a bacterial surface display library and said

12. Method according to any one of claims 1 to 11, characterized in that said produced hyperimmune serum-reactive antigens are finished to a pharmaceutical preparation, optionally by a

13. Method according to claim 12, characterized in that said pharmaceutical preparation is a vaccine.

14. Method according to claim 12 or 13, characterized in that said pharmaceutically acceptable carrier and/or excipient is an immunostimulatory compound.

15. Method according to claim 14, characterized-in that said immunostimulatory compound is selected from the groupof polycati- onic substances, especially polycationic. peptides, imn

16. Method according to any one of claims 1to 15, characterized in that said individual antibody preparations are derived from patients with acute infection with said pathogen, especially

17. Method according to any one of claims 1 to 16, characterized in that at least 10, preferably at least 30, especially at least 50, individual antibody preparations are used in identifying sa

18. Method according to any one of said claims 1 to 17, characterized in that said relevant portion of said individual antibody preparations from said individual sera are at least 10, prefera

19. Method according to any one of claims 1 to 18, characterized in that said individual sera are selected by having an IgA titer against a lysate, cell wall components or recombinant prote

20. Method according to any one of claims 1 to 19, characterized in that said pathogen is a Staphylococcus pathogen, especially
Staphylococcus aureus. and/or Staphylococcus epidermidis.

21. A hyperimmune serum-reactive antigen selected from the group consisting of the sequences listed in any one of Tables 2a, 2b, 2c, 2d, 3,4 and 5, especially selected from the group cor
fragments thereof.

22. A hyperimmune serum-reactive antigen obtainable by a method according to any one of claims 1 to 20 and being selected from the group consisting of the sequences listed in any one
120,122,126,128,130,132,134,138,140,142,151,152,154, 155 and hyperimmune fragments thereof.

23. Use of a hyperimmune serum-reactive antigen selected from the group consisting of the sequences listed in any one of Tables 2a,2b, 2c, 2d, 3,4 and 5, especially selected from the gro
138,140,142.,151, 152,154,155,158 and hyperimmune fragments thereof for the manufacture of a pharmaceutical preparation, es pecially for the manufacture of a vaccine against staphylc

24. Hyperimmune fragment of a hyperimmune serum-reactive antigen selected from the group consisting of peptides comprising the amino acid sequences of column"predicted immunog
amino acid No. aa 12-29, 34-40,63-71,101-110,114-122,130-138,140-195,197-209,215229,239-253,255-274 and 39-94 of Seq. ID No. 55, aa 5-39,111-117,125-132,134-141,167-191,196
702-715,723-731,786-793,805-811,826-839,874-889,37-49,6377 and 274-334, of Seq. ID No. 56, aa 28-55,82-100,105-111,125-131,137-143,1-49, of Seq. ID No.

57, aa 33-43,45-51,57-63,65-72,80-96,99-110,123-129,161-171, 173-179,185-191,193-200,208-224,227-246,252-258,294-308, 321-329,344-352,691-707,358-411 and 588-606, of Seq. I
18-23,42-55,69-77,85-98,129-136,182-188,214-220,229235,242-248,251-258,281-292,309-316,333-343,348-354,361367,393-407,441-447,481-488,493-505,510-515, 517-527,530535,5
1122-1129,1134-1143,1180-1186,1188-1194,1205-1215,1224-1230, 1276-1283,1333-1339,1377-1382,1415-1421,1448-1459,1467-1472, 1537-1545,1556-1566,1647-1654,1666-1675,1
2232-2243,198-258,646-727 and 2104-2206, of Seq. ID No. 60, aa 10-29,46-56,63-74,. 83-105,107-114,138-145,170-184,186193,216-221,242-248,277-289,303-311,346-360,379-389,42

62, aa 14-22,32-40,52-58,61-77,81-93,111-117,124-138,151-190, 193-214,224-244,253-277,287-295,307-324,326-332,348-355, 357-362,384-394,397-434,437-460,489-496,503-510,51
No. 66, aa 49-56,62-68,83-89,92-98,109-115,124-131,142-159,161167,169-175,177-188,196-224,230-243,246-252,34-46, of
Seq. ID No. 67, aa 11-20,26-47,69-75,84-92,102-109,119-136,139-147,160170,178-185,190-196,208-215,225-233,245-250,265-272,277284,300-306,346-357,373-379,384-390,429-435,
6-20,53-63,83-90,135-146,195-208,244-259,263-314,319327,337-349,353-362,365-374,380-390,397-405,407-415,208287 and 286-314, of Seq. ID No. 71, aa 10-26,31-43,46-58,61-66,6

128-135, 149-155,167-173,178-187,189-196,202-222,225-231, 233-240,245-251,257-263,271-292,314-322,325-334,339-345, 59-74, of Seq. ID No. 72, aa 4-9,15-26,65-76,108-115,119-
10-26,31-44,60-66,99-104,146-153,163-169,197-205,216223,226-238,241-258,271-280,295-315,346-351,371-385,396407,440-446,452-457,460-466,492-510,537-543,546-551,565582,5
ID No. 77, aa 5-24,88-94,102-113,132-143,163-173,216-224,254-269,273 278,305-313,321-327,334-341,31-61 and 58-74, of Seq. ID No.

78, aa 16-24,32-39,43-49,64-71,93-99,126-141,144-156,210-218, 226-233,265-273,276-284,158-220, of Seq. ID No. 79, aa 49-72,76-83,95-105,135-146,148-164,183-205,57-128, of
Seq. ID No. 80, aa 6-15,22-32,58-73,82-88,97-109,120-131,134-140,151-163, 179-185,219-230,242-255,271-277,288-293,305-319,345-356, 368-381,397-406,408-420,427-437,448-454,
916-921,929-935,949-955,1001-1008,1026-1032,1074-1083,10881094,1108-1117,1137-1142,1159-1177,1183-1194,1214-1220, 1236-1252,1261-1269,1289-1294,1311-1329,1336-1341,
Seq. ID No. 81, aa 6-33,40-46,51-59,61-77,84-104,112-118,124-187,194-248, 252-296,308-325,327-361,367-393,396-437,452-479,484-520, 535-545,558-574,582-614,627-633,656-663,
4-19,57-70,79-88,126-132,144-159,161-167,180-198,200212,233-240,248-255,276-286,298-304,309-323,332-346,357366,374-391,394-406,450-456,466-473,479-487,498-505,507519,5
1105-1116,1124-1135,1144-1151,1173-1181,1186-1191,1206-1215, 1225-1230,1235-1242,6-66,65-124 and 590-604, of Seq. ID No. 83, aa 5-32,66-72,87-98,104-112,116-124,128-137,1
183,248-254,261-266,289-303,312-331,174-249, of Seq. ID No.

84, aa 4-21,28-40,45-52,59-71,92-107,123-137,159-174,190-202,
220-229,232-241,282-296,302-308,312-331,21-118, of Seq. ID
No. 85, aa 9-28,43-48,56-75,109-126,128-141,143-162,164-195,197
216,234-242,244-251,168-181, of Seq. ID No. 87, aa 4-10,20-42,50-86,88-98,102-171,176-182,189-221,223-244, 246-268,276-284,296-329,112-188, of Seq. ID No. 88, aa 4-9,13-24,26-
Seq. ID No. 90, aa 26-53,95-123,164-176,189-199,8-48, of Seq.ID No. 92, aa 7-13,15-23,26-33,68-81,84-90,106-117,129-137,140-159, 165-172,177-230,234-240,258-278,295-319,22-5
46-80,92-98,105-113,118-123,133-165,176-208,226238,240-255,279-285,298-330,338-345,350-357,365-372,397402,409-415,465-473,488-515,517-535,542-550,554-590,593601,603-6
Seq. ID No. 99, aa 5-12,15-20,43-49,94-106,110-116,119-128,153-163,175180,185-191,198-209,244-252,254-264,266-273,280-288,290297,63-126,of Seq. ID No. 100, aa 5-44,47-55,62
7-37,56-71,74-150,155-162,183-203,211-222,224-234,242272,77-128, of Seq. ID No. 103, aa 34-58,63-69,74-86,92-101,130-138,142-150,158-191,199207,210-221,234-249,252-271,5-4
61-75,82-87,97-104,113-123,128-133,203-216,224-229, 236-246, 251-258,271-286,288-294,301-310,316-329,337-346, 348-371,394-406,418-435,440-452 of Seq. ID No. 112, aa 30-37,
321-326,338-345,360-369,385-391 of Seq. ID No. 116, aa 9-33,56-62,75-84,99-105,122-127,163-180,186-192,206228,233-240,254-262,275-283,289-296,322-330,348-355,416424,426-

443-449,497-503,505-513,539-545,552-558,601-617,629-649, 702-711,736-745,793-804,814-829,843-858,864-885,889-895, 905-913,919-929,937-943,957-965,970-986,990-1030,1038
149-155,159-171,180-185,189-209,228-234,245-262,264-275, 280-302,304-330,343-360,391-409,432-437,454-463,467-474, 478-485,515-528,532-539,553-567,569-581,586-592,605-6

154, aa 13-28,40-46,69-75,86-92,114-120,126-137,155-172,182193,199-206,213-221,232-238,243-253,270-276,284-290,22100, of Seq. ID No. 155 and aa 7-19,46-57,85-91,110-117,12
5-24,38-44,100-106,118-130,144-154,204-210,218-223,228243,257-264,266-286,292-299 of Seq. ID. No. 174, aa 29-44,74-83,105-113,119-125,130-148,155-175,182-190,198-211, 238-
Tables 2 and 4 and fragments comprising at least 6, preferably more than 8, especially more than 10 aa, of said sequences.

25. Helper epitopes of an antigen or a fragment, as defined in anyone of claims 21 to 24, especially peptides comprising fragments selected from the peptides mentioned in column"Putati
Seq. ID. No. 70, aa 240-260 of Seq. ID. No. 74, aa 1660-1682 and 17461790 of Seq. ID. No. 81, aa 1-29,680-709, and 878-902 of
Seq. ID. No. 83, aa 96-136 of Seq. ID. No. 89, aa 1-29,226-269 and 275-326 of Seq. ID. No. 94, aa23-47 and 107-156 of Seq. ID. No. 114 and aa 24-53 of Seq. ID. No. 142 and fragment

26. Vaccine comprising a hyperimmune serum-reactive antigen or a fragment thereof, as defined in any one of claims 21 to 25.

27. Vaccine according to claim 25, characterized in that it further comprises an immunostimulatory substance, preferably selected from the group comprising polycationic polymers, espec

28. Preparation comprising antibodies against at least one antigen or a fragment thereof, as defined in any one of claims 21 to 25.

29. Preparation according to claim 27, characterized in that said antibodies are monoclonal antibodies.

30. Method for producing a preparation according to claim 28, characterized by the following steps: initiating an immune response in a non human animal by admin istering an antigen or anti gen and producing the antibody preparation by cultivation of said cloned hybridoma cells and optionally further purification steps.

31. Method according to claim 29, characterized in that said removing the spleen or spleen cells is connected with killing said animal.

32. Method for producing a preparation according to claim 27, characterized by the following steps: initiating an immune response in a non human animal by admin istering an antigen or purification steps.

33. Use of a preparation according to claim 27 or 28 for the manufacture of a medicament for treating or preventing staphylococcal infections or colonization in particular against Staphyl

, 34. A screening method assessing the consequences of functional inhibition of at least one antigen or a fragment thereof, as defined in any one of claims 21 to 25.